J. Anat (1989), **164**, pp. 175–187
With 24 figures
Printed in Great Britain

The fine structure of initial mineralisation during tooth development in the gummy shark, *Mustelus manazo*, Elasmobranchia*

ICHIRO SASAGAWA

Department of Anatomy, School of Dentistry at Niigata, The Nippon Dental University, 1–8 Hamaura-cho, Niigata 951, Japan

(*Accepted* 13 *September* 1988)

INTRODUCTION

The major force for work on the fine structure of tooth germs in sharks has been on the epithelial components, especially the inner dental epithelial (IDE) cells and their functions (Kemp & Park, 1974; Kerebel, Daculsi & Renaudin, 1977; Goto, 1978; Nanci, Bringas, Samuel & Slavkin, 1983; Kemp, 1985). These authors suggested that the IDE cells in tooth germs of sharks markedly influence their enameloid formation and that they secrete the organic matrix into the enameloid area. By contrast, little is known about the fine structure of the odontoblasts and the initial mineralisation in the tooth germs of sharks. The initial mineralisation sites in sharks have been found in the enameloid area near the apex and along the cutting edges (Kerr, 1955; Fosse, Risnes & Holmbakken, 1974). Preceding work by Garant (1970), with the dogfish, *Squalus acanthias*, showed that, initially, crystals appear in the small membranous saccules which constitute the enameloid matrix in the tooth germs. In the teleost, the initial site of mineralisation is the junction between the enameloid and dentine (Kerr, 1960; Isokawa *et al.* 1970). Although the matrix vesicles may play an important role in the initial mineralisation at this junction, further mineralisation, which progresses both towards the enameloid and dentine side, is mainly composed of depositions of crystals along the collagen fibrils in teleost fishes which have well-developed enameloid and orthodentine (Sasagawa, 1988). Hence, I believe that the mechanism for initial mineralisation is quite different in sharks and teleosts. This study elucidates the peculiar manner in which initial mineralisation occurs and the participation of the odontoblasts in the mineralisation in the tooth germs of the gummy shark.

MATERIALS AND METHODS

Four adult gummy sharks, *Mustelus manazo* (total length 41, 43, 53 and 69 cms) were collected from the Japan Sea and used in this study.

After decapitation, the tooth-bearing jaws were dissected out and placed in Karnovsky's fixative (0·1 N cacodylate buffer added to 1·7% NaCl, pH 7·4) for 9–12 hours at room temperature followed by a 1% osmium tetroxide solution buffered with cacodylate. Selected specimens were demineralised with 2·5% EDTA-2Na or 2% L-ascorbic acid solution (Wakita, Kobayashi & Shioi, 1983) after initial fixation. After dehydration, the specimens were embedded in Araldite-Epon resin. Semithin sections were cut with glass knives, stained with toluidine blue and examined using a light microscope. Ultrathin sections were cut with diamond knives, stained with uranyl

* Reprint requests to I. Sasagawa, Department of Cell and Structural Biology, School of Biological Sciences, University of Manchester, Coupland III Building, Coupland Street, Manchester M13 9PL (until August, 1989).

acetate and lead citrate, or lead citrate only and examined using a H-500 TEM or a JEOL-1200EX TEM.

RESULTS

The enameloid matrix formation stage

This stage, which probably corresponds to the 'cone-shaped teeth before mineralisation' stage of Kemp & Park (1974), Stages II and III of Goto (1978) and Row II of Nanci *et al.* (1983), constitutes the phase when the enameloid matrix appears between the IDE cells and odontoblasts.

In the early period of this stage a large number of odontoblast processes occupied the area beneath the IDE cells (Fig. 1). These elongated processes were gathered together and formed ill-defined palisades (Garant, 1970), the processes running almost parallel to the basal lamina which is adjacent (Fig. 2). Diverticula from the processes were often observed. In the odontoblast processes there were many microtubules and microfilaments aligned parallel to the long axis of the processes along with free ribosomes, rough surfaced endoplasmic reticulum (ER) and small vesicles (Fig. 3), but few lysosomes and mitochondria were seen. Short gap-like junctions were seen between the processes. Many rER, mitochondria and widely distributed Golgi apparatus were contained in the cytoplasm of the odontoblasts, and many microtubules and microfilaments were found in the cytoplasm near the nuclei (Fig. 4).

A large number of fine and electron-dense tubular vesicles were found around the odontoblast processes (Fig. 2). These initially appeared in the enameloid area near the apex of the tooth and beneath the basal lamina. Aggregations of tubular vesicles near the apex were far more numerous than those found beneath the basal lamina (Fig. 5). The initial distribution of the tubular vesicles corresponded to the initial mineralisation sites in the tooth germs reported by Kerr (1955) and Fosse *et al.* (1974). There were a few odontoblast processes but many large vacuoles were found in the large aggregation of tubular vesicles. The tubular vesicles were 15–20 nm thick and appeared fibre-like. The tubular vesicles often branched out and seemed to form an entwining network. The cross-sections of these tubular vesicles were round and were limited by the trilaminar unit membrane. The contents of the tubular vesicles were rather electron-lucent (Fig. 6). The tubular vesicles often came into contact with the odontoblast processes and in such cases, the membrane of the tubular vesicles seemed to connect with that of these processes (Fig. 6, inset; Fig. 7). Several electron-dense granules were clearly observed in the tubular vesicles in undemineralised specimens stained with lead citrate only (Fig. 8), although they were indistinct in the specimens stained with uranyl acetate and lead citrate (Figs. 6, 7).

In the late period of this stage, the tubular vesicles occupied the majority of the

---

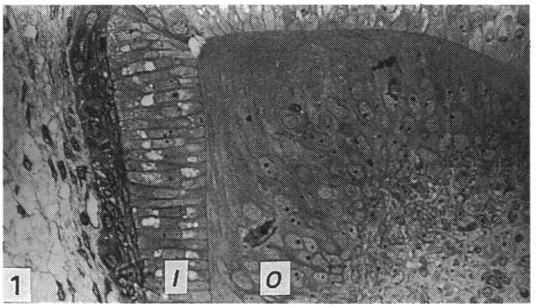
Fig. 1. A tooth germ in the early enameloid matrix formation stage. *I*, IDE cells; *O*, odontoblasts. Semithin section stained with toluidine blue. ×223.

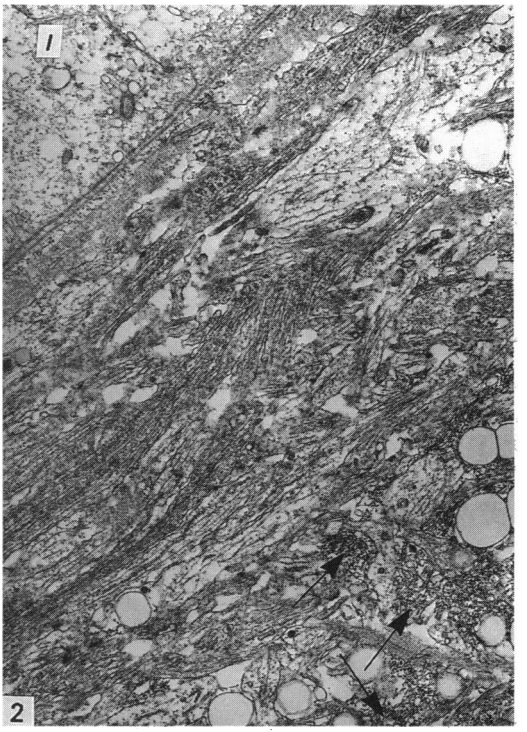
Fig. 2. A large number of the odontoblast processes occupying the dental papilla near the IDE cells. The same tooth germ as Fig. 1. *I*, an IDE cell. Arrows indicate the aggregation of tubular vesicles. Non-demineralised section stained with uranyl acetate and lead citrate (U–Pb) ×7100.

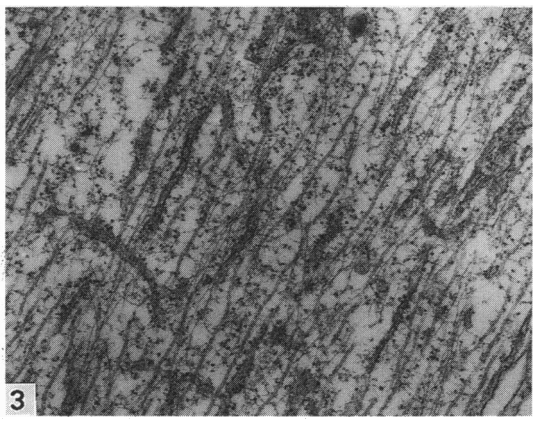
Fig. 3. Enlarged view of an odontoblast process. Microtubules and microfilaments showing their parallel alignment to the long axis of the processes. Non-demineralised, U–Pb. ×17800.

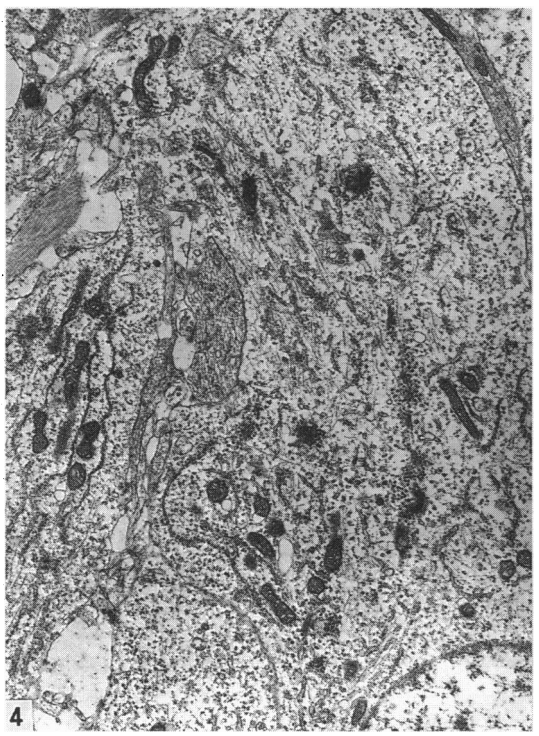
Fig. 4. Cytoplasm around the nucleus of an odontoblast. The same tooth germ as Fig. 1. Non-demineralised, U–Pb. ×5680.

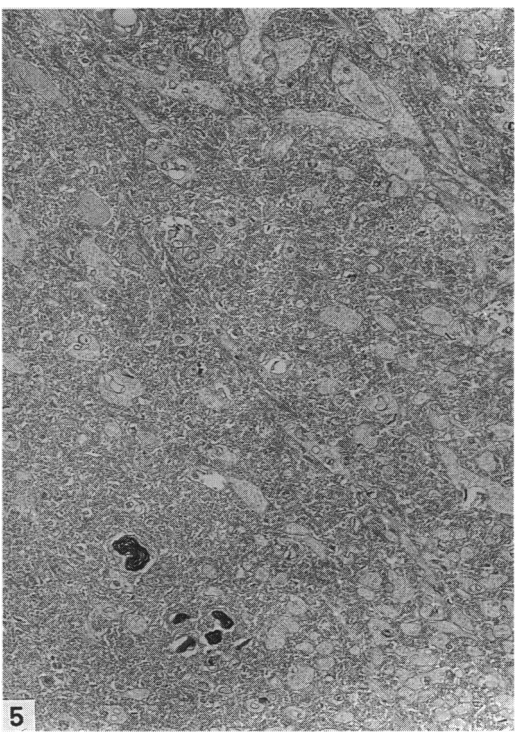
Fig. 5. Section of tooth germ with a large aggregation of tubular vesicles near the apex. Cross-sections of odontoblast processes are scattered among the tubular vesicles. The number of vacuoles had not as yet increased. Demineralised with EDTA, U–Pb. ×5680.

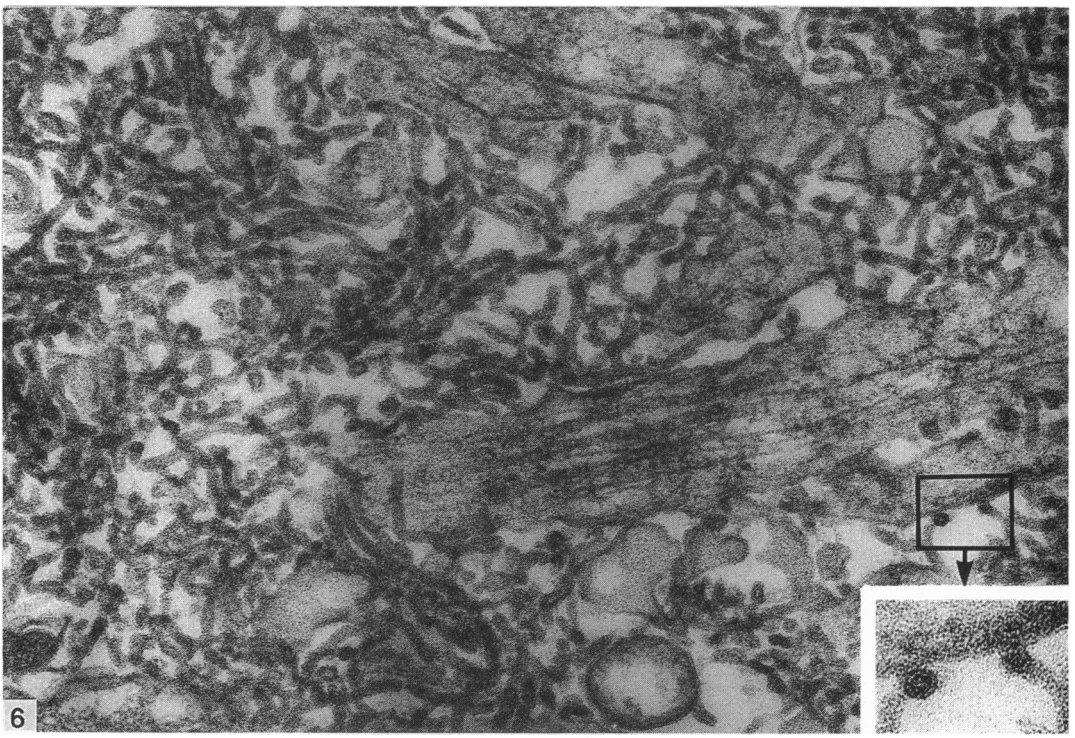

1
I
O


I
2


3


4


5



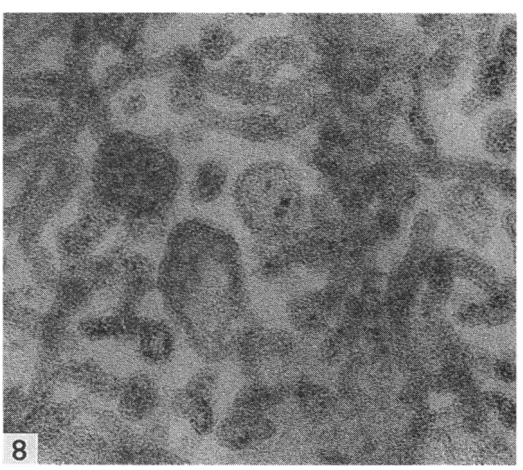
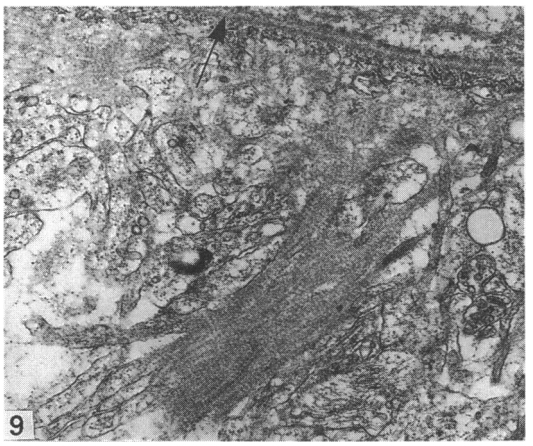
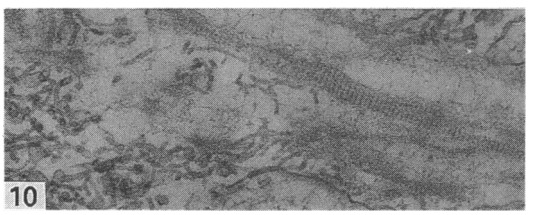


Fig. 6. Enlarged view of odontoblast processes and tubular vesicles. Non-demineralised, U–Pb. ×64200. Inset shows the area outlined by the square, ×140210.
Fig. 7. Enlarged view of odontoblast processes and tubular vesicles. An arrow indicates a tubular vesicle which appears to be connected to an odontoblast process. Non-demineralised, U–Pb. ×118055.
Fig. 8. Tubular vesicles showing many electron-dense granules. Non-demineralised, lead citrate only. ×177777.

enameloid matrix. Among the tubular vesicles there were vacuoles, vesicles, secondary lysosomal granules, flocculent material and fibrils. The fibrils were of two types: collagen fibrils showing cross-banding with a periodicity of 40–60 nm (Fig. 9) and electron-dense fibrils showing cross-banding with a periodicity of about 15 nm (Fig. 10, 'giant fiber' of Kemp & Park, 1974). These fibrils were mainly arranged vertically





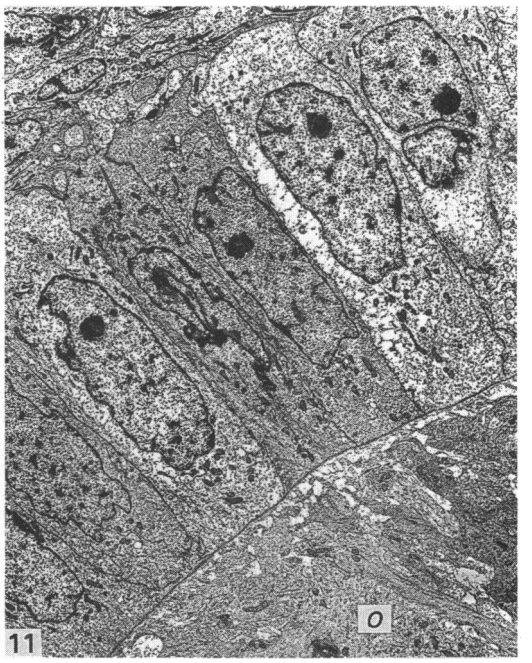
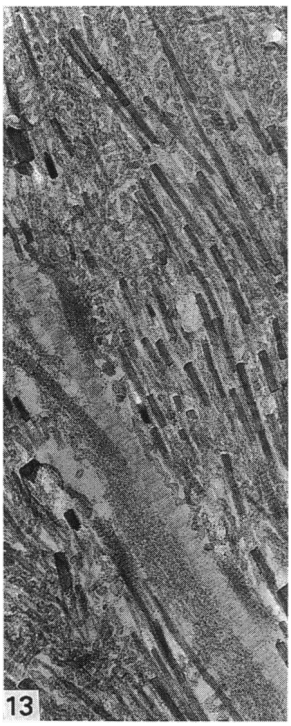


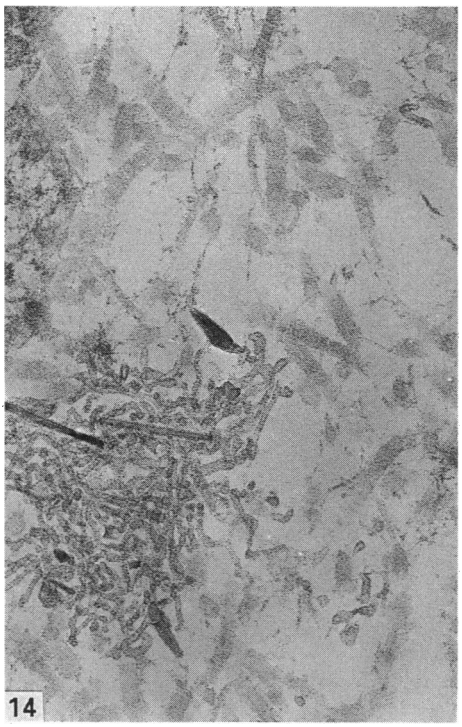
Fig. 9. Collagen fibrils showing periodic bands (40–60 nm). An arrow indicates the basal lamina beneath the IDE cells. Non-demineralised, U–Pb. ×10680.

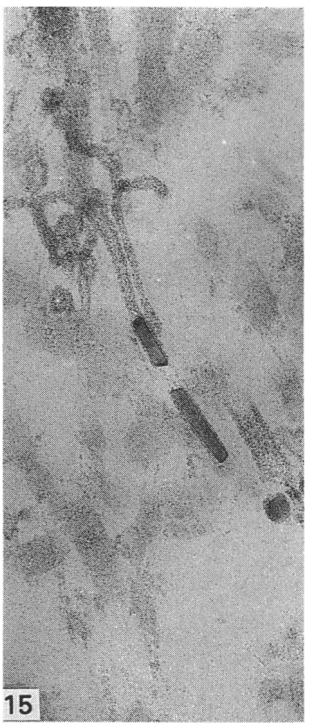
Fig. 10. Fibrils having a short periodic band (15nm). Demineralised with EDTA, U–Pb. ×35600.

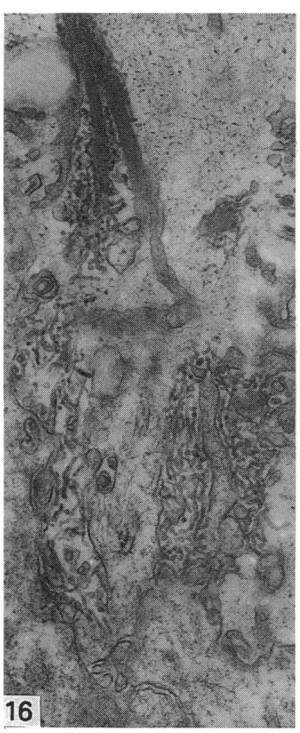
Fig. 11. IDE cells in the stage of enameloid matrix formation. This is the same tooth germ as shown in Fig. 1. *O*, a part of an odontoblast. Non-demineralised U–Pb. ×2270

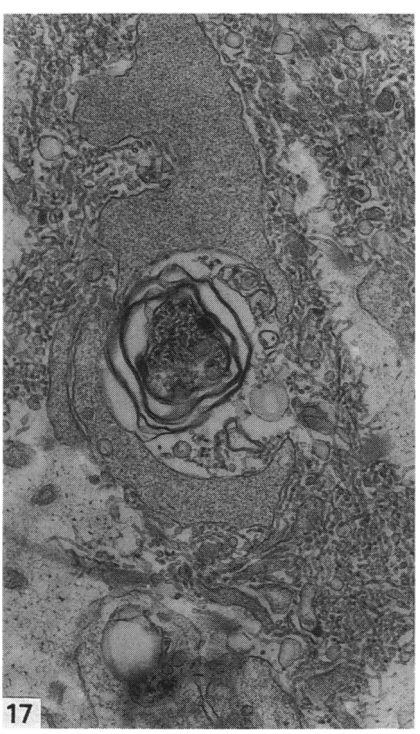
Fig. 12. Enlarged view of tubular vesicles and initial crystals in the stage of mineralisation. Non-demineralised, U–Pb. ×213888. Inset, a section demineralised with L-ascorbic acid. U–Pb. ×177777.

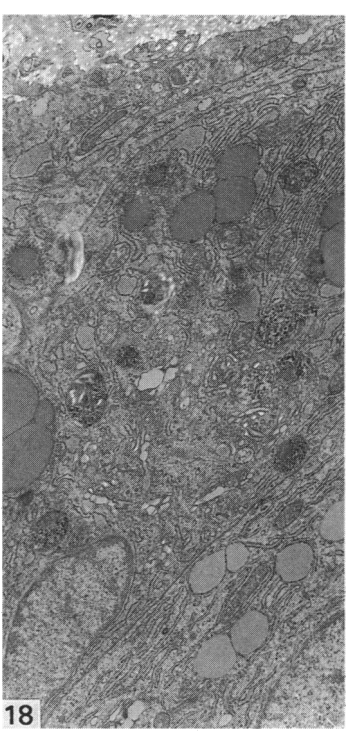

13


14


15


16


17


18

to the basal lamina and were usually seen in the sparse enameloid matrix between large aggregations of tubular vesicles (palisades) and beneath the basal lamina.

Although the IDE cells were columnar, there were only a few mitochondria, vesicles and vacuoles in the distal cytoplasm (Fig. 11). The basal lamina was defined and the lamina densa was uniformly homogenous and 40–50 nm thick (Fig. 9).

The mineralisation stage

In this stage crystals appeared in the enameloid matrix. The dentine matrix had formed but mineralisation of dentine had not yet started. This stage probably corresponds to the 'early stage of mineralisation of the enamel layer' of Kemp & Park (1974), Stage IV and V of Goto (1978) and Row III of Nanci *et al* (1983).

The number of vesicles and lysosomal bodies increased in the aggregation of tubular vesicles (palisades) constituting the majority of the enameloid matrix. Enameloid crystals appeared in the tubular vesicles. Initially the crystals were long and 15–20 nm wide which was similar to that of the tubular vesicles. The unit membrane surrounding each crystal continued into that of the tubular vesicles (Fig. 12). The cross-sections of crystals displayed a hexagonal shape (Fig. 12, inset). Although there were two kinds of fibrils in the enameloid matrix, no crystals were observed along these fibrils at this stage (Fig. 13). The aggregation of tubular vesicles disappeared at the dentine-enameloid junction (Fig. 14) and only large crystals, contained in the tubular vesicles, were found in this region (Fig. 15).

The unmineralised dentine matrix consisted chiefly of collagen fibres, which displayed an irregular arrangement, and flocculent material. There were a few odontoblast processes and small aggregations of tubular vesicles (Fig. 14, right side). Electron-dense fibrils having short periodic bands at intervals of about 15 nm were sometimes seen in the dentine matrix. The aggregations of tubular vesicles came into contact with the odontoblast processes in the unmineralised dentine (Fig. 16). Some aggregations of tubular vesicles were surrounded by parts of these processes (Fig. 17). Multivesicular bodies were present in which the contents resembled the tubular vesicles and secondary lysosomes were found in the odontoblast processes. Well-developed Golgi apparatus, many mitochondria, expanded rER containing dense materials, lysosomal bodies and various vesicles were observed in the cytoplasm around the nuclei of odontoblasts. Many multivesicular bodies were present near the Golgi apparatus (Fig. 18).

In the distal cytoplasm of IDE cells there were scattered mitochondria, smooth-surfaced endoplasmic reticulum (sER), vesicles, vacuoles and free ribosomes. Although the small processes of lateral cell membrane (interdigitations) were present between each neighbouring IDE cell, the distal cell membranes were more or less

---

Fig. 13. Enameloid in the stage of mineralisation. Two kinds of fibrils but no crystals are visible. Non-demineralised, U–Pb, ×45416.

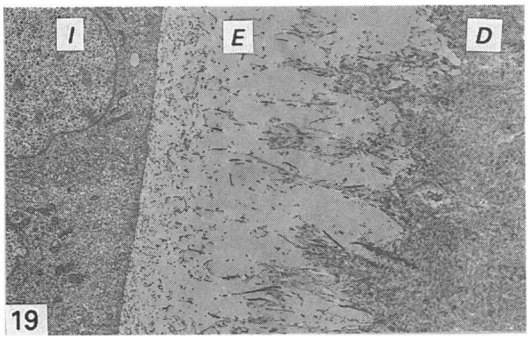
Fig. 14. Enlarged view of the junction between enameloid and unmineralised dentine. Non-demineralised, U–Pb. ×45833.

Fig. 15. Tubular vesicles containing crystals seen at the dentine-enameloid junction. Non-demineralised, U–Pb. ×91666.

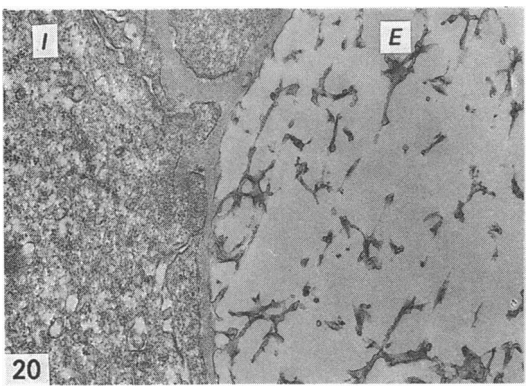
Fig. 16. Odontoblast processes with tubular vesicles around them. Demineralised with EDTA, U–Pb. ×21300.

Fig. 17. Odontoblast processes surrounding a small aggregation of tubular vesicles. Demineralised with EDTA, U–Pb. ×21300.

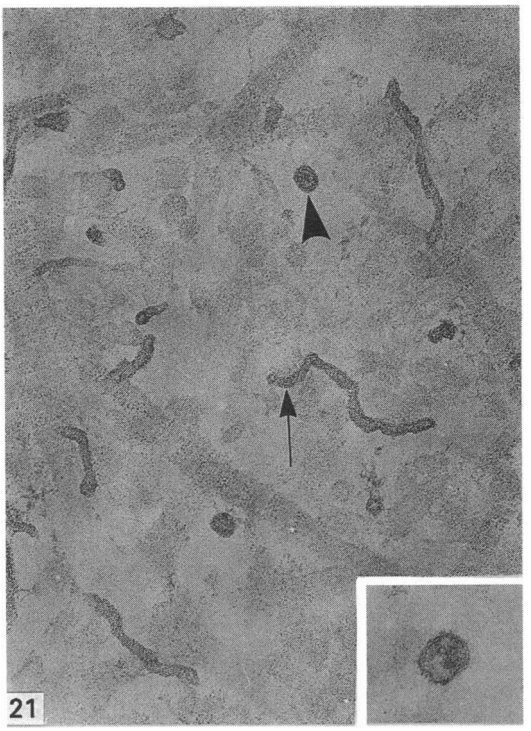
Fig. 18. Odontoblasts in the stage of enameloid mineralisation. Demineralised with EDTA, U–Pb. ×6000.

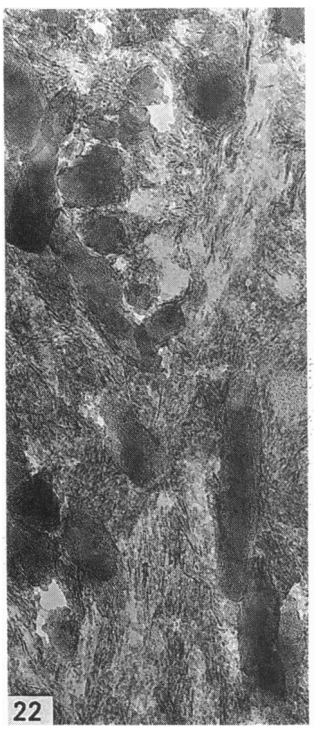


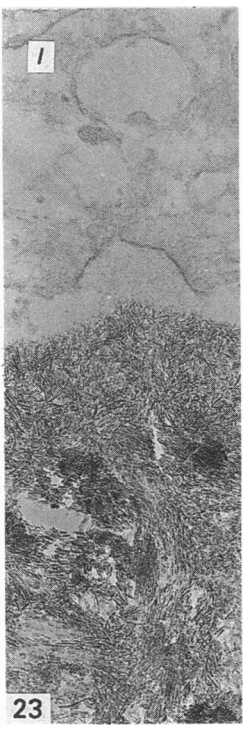
19



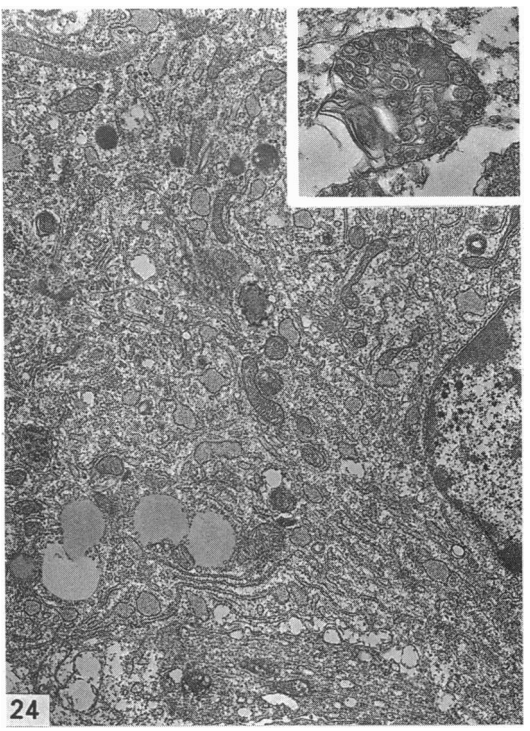
20


21


22



23


24

straight except for small pinocytotic pits. The basal lamina was well-defined and showed a thick lamina densa.

The maturation stage

In this stage, crystals in the enameloid grew rapidly and the degree of mineralisation increased. The mineralisation of dentine started so that the distinction between the dentine and predentine was defined. This stage probably corresponds to Stage VI and VII of Goto (1978) and Row IV of Nanci *et al.* (1983).

After demineralisation with EDTA scattered vesicles, vacuoles, flocculent materials, fragments of unit membrane and fibrils having short (about 15 nm) periodic bands were observed in the enameloid. The dentine-enameloid junction was extremely irregular and intricate (Figs. 19, 20). In the predentine, the odontoblast processes, some matrix vesicles, isolated tubular vesicles and fragments of fibrils having short periodic bands were found (Fig. 21). Matrix vesicles were 30–120 nm in diameter and clearly larger than the cross-sections of tubular vesicles (Fig. 21, inset). The contents of matrix vesicles contained somewhat electron-dense material, but they and the tubular vesicles contained no crystals. In the dentine, fine crystals formed irregular aggregations, although several crystals seemed to be arranged along the collagen fibres. The fine crystals were found among the large enameloid crystals at the junction of the dentine-enameloid (Fig. 22). At the basal side of the tooth germs, by contrast, the fine crystals filled the area beneath the basal lamina and large enameloid crystals lay scattered in the mass of fine crystals (Fig. 23).

Well-developed Golgi apparatus and other organelles were observed in the cytoplasm around the nuclei of odontoblasts. Many large multivesicular bodies were particularly noticeable in the cytoplasm on the distal aspect of the Golgi area (Fig. 24).

The number of organelles increased in the distal cytoplasm of the IDE cells in this stage and there were a number of mitochondria, vesicles and sER. The small interdigitating processes of the lateral cell membrane were more numerous than in the previous stage, although the distal cell membrane continued to be almost straight. The basal lamina was generally clearly seen throughout almost all its length but there were some areas (Fig. 20) where it could not be seen.

DISCUSSION

In this study, the progress of initial mineralisation was as follows.

(i) A large number of tubular vesicles limited by unit membrane appeared from the early enameloid matrix formation stage.

Fig. 19. A part of the tooth germ in the stage of maturation. *I*, IDE cells; *E*, enameloid: *D*, dentine. Demineralised with EDTA, U–Pb. ×3027.

Fig. 20. Enlarged view of surface area enameloid. *I*, IDE cells; E, enameloid. Demineralised with EDTA, U–Pb. ×17777.

Fig. 21. Enlarged view of predentine. An arrowhead indicates a matrix vesicle and an arrow indicates a tubular vesicle. Demineralised with EDTA, U–Pb. ×75694. Inset, an enlarged matrix vesicle. Non-demineralised, U–Pb. ×90833.

Fig. 22. Enlarged view of the dentine-enameloid junction. Non-demineralised, U–Pb. ×75694.

Fig. 23. Fine crystals filling the area beneath the basal lamina at the basal side of tooth germ. *I*, an IDE cell. Non-demineralised, U–Pb. ×60555.

Fig. 24. Cytoplasm around a nucleus of an odontoblast in the stage of enameloid maturation. Demineralised with EDTA, U–Pb. ×7569. Inset, an enlarged multivesicular body. ×22708.

(ii) Many electron-dense granules, thought to be precursors of the crystals in the tubular vesicles, were found in undemineralised sections stained with lead citrate only.

(iii) Initial enameloid crystals appeared in the tubular vesicles. These crystals were similar in diameter to the tubular vesicles.

(iv) Unit membrane surrounded the initial enameloid crystals and continued into that of the tubular vesicles.

In the tooth germs of *Mustelus manazo*, the initial enameloid crystals were formed in the tubular vesicles which occupy the majority of the enameloid matrix. The tubular vesicles in this study are probably identical to the 'small membranous saccules' (Garant, 1970), 'enameline fibrils (tubules)' (Kemp & Park, 1974; Kemp, 1985) and 'tubular structures' (Goto, 1978) found in other sharks. However, previous workers, with the exception of Garant, have not referred to the unit membrane limiting these tubular structures.

Although Garant (1970) made no interpretation of the origin of the membranous saccules, other workers have suggested that the fine tubular structures in the enameloid matrix originate from the IDE cells (Kemp & Park, 1974; Goto, 1978; Kemp, 1985). Kemp mentioned that the tubular sheath surrounding the enameloid crystals in the shark is similar to the amelogenin and/or enamelin in mammalian enamel. I believe, however, that the tubular vesicles in the enameloid matrix in this study originated from the odontoblasts, chiefly the processes of them, for the following reasons. (i) The tubular vesicles initially appeared among the odontoblast processes. (ii) The vesicles often came into contact with the odontoblast processes and the membrane of the tubular vesicles seemed to connect with that of the processes. (iii) The odontoblasts in the early period of enameloid matrix formation seemed to be active cells, because they possessed many processes containing a number of microtubules and microfilaments and had well-developed organelles around the nuclei.

Prostak, Seifert & Skobe (1988*a*, *b*) suggested that the odontoblasts are actively engaged in secretion during enameloid matrix formation in the skate. By contrast, the IDE cells had scattered organelles and seemed less active than the odontoblasts although the IDE cells were columnar in the early enameloid matrix formation stages. It appears unlikely, in the shark, that a large number of enamel proteins produced by the IDE cells could pass through the basal lamina, contribute to unit membranes in the enameloid area and then become tubular vesicles. The 4·8 nm-wide wall of the tubular organic sheaths, which are resolved into two dense strands in developing bovine enamel (Travis & Glimcher, 1964), seems to be different from the trilaminar unit membrane composed of cell membrane and this wall is slightly narrower than the membrane of the tubular vesicles in this study.

Some reports concerning immunohistochemical methods suggest that amelogenin-like materials originating from the IDE cells exist in the enameloid matrix of shark (Herold, Graver & Christher, 1980; Slavkin *et al.* 1983). However, immunofluorescent staining has been detected in the enameloid at the stage of mineralisation (Row III, Slavkin *et al.* 1983). Moreover, the tallest IDE cells, having the most well-developed organelles, were found from the late mineralisation stage to the maturation stage (Stages V and VI of Goto, 1978; Rows III and IV of Nanci *et al.* 1983). The stages mentioned above do not correspond to the period in which a large number of tubular vesicles appear in the enameloid matrix. If the IDE cells secrete the amelogenin-like materials into the enameloid, they may have a role in maturation other than initial mineralisation.

In the mineralisation stage, some aggregations of tubular vesicles were surrounded by a part of the odontoblast processes in the dentine matrix. There were many multivesicular bodies, in which the contents resembled the tubular vesicles and lysosomes in the cytoplasm of odontoblasts from the mineralisation stage to the maturation stage. Only a few separate tubular vesicles were seen in the predentine during the maturation stage. It appears, that after the beginning of dentine formation, the odontoblasts absorb and break the tubular vesicles.

There are many differences found between the initial mineralisation stages of sharks and teleosts. In teleosts, mineralisation of tooth germs begins initially at the junction between the enameloid and dentine. Matrix vesicles are probably involved directly in the initial mineralisation (Sasagawa, 1988). In this study of sharks, crystals appeared initially in the tubular vesicles situated in the apical enameloid matrix and no matrix vesicles were found in the enameloid matrix. Although fine needle-like crystals constitute the initial mineralisation site in teleosts (Sasagawa, 1988), the initial crystals of the shark are larger and grow rapidly into large hexagonal crystals and initially no fine needle-like crystals are found in the enameloid. Additional mineralisation occurred along the collagen fibrils after the initial mineralisation in the enameloid of teleosts (Sasagawa, 1988). In sharks, conversely, the collagen fibres in enameloid seemed not to be connected with the mineralisation process (Kerebel *et al.* 1977). These facts are at variance with the opinion that the mechanism of formation of enameloid in sharks is similar to that in teleosts (Reif, 1979). Therefore, it seems that the enameloid in sharks is essentially a hard tissue distinct from that found in teleosts (Bendix-Almgreen, 1983). Moreover, a histochemical study has suggested that the enameloid in shark is a particular hard tissue which is distinct from dentine and mammalian enamel (Everett & Miller, 1981). Even if there are amelogenin-like materials originating from the IDE cells in shark enameloid, the opinion that the shark enameloid is homologous with mammalian enamel (Moss, 1977) is not acceptable because of the characteristics of shark enameloid mentioned above. Shark enameloid, teleost enameloid and mammalian enamel, although similar in final form, each have peculiar evolutionary development. At the present time, they can be said to be distinct hard tissues and have different mechanisms of formation.

Kemp & Westrin (1979) described a few matrix vesicles around chondrocytes in the shark. This study also showed a small number of matrix vesicles in the predentine. However, these matrix vesicles were not an element of mineralisation because they contained no crystals. The mineralisation along the collagen fibres is probably principally in the dentine of sharks as suggested by Garant (1970). I believe that the matrix vesicles are involved directly with the initial mineralisation of tooth germs in the vertebrates which are evolutionarily higher than the sharks. I also assume that although the tubular vesicles in the enameloid matrix of sharks are similar to matrix vesicles within the limits of vesicles restricted by unit membrane and derived from the odontoblasts, the differences of initial crystals between the tubular vesicles and matrix vesicles suggest a distinct nature for each type of vesicle.

SUMMARY

In the enameloid matrix a large number of tubular vesicles appeared around the odontoblast processes at the stage of enameloid matrix formation. The tubular vesicles were limited by unit membrane and the membrane often seemed to connect with that of the odontoblast processes. The tubular vesicles probably originated from the odontoblasts. Electron-dense granules were observed in the tubular vesicles before the

appearance of crystals and then, in the stage of mineralisation, initial enameloid crystals appeared in each tubular vesicle. Parts of the odontoblast process surrounded small aggregations of tubular vesicles in the unmineralised dentine matrix. There were many multivesicular bodies and lysosomes in the odontoblasts forming the dentine. The odontoblasts probably absorb and break down the tubular vesicles after the beginning of dentinogenesis. Only in the predentine, during the enameloid maturation stage, did a few matrix vesicles appear, but no crystals were found in them. Hence, the initial mineralisation in tooth germs of the shark can be said to be different from that in teleosts.

I wish to thank Professor Yoshiharu Honma, Dr Takehiko Kitami and Mr Yukihiro Toda, Department of Biology, Faculty of Science, University of Niigata, for their valuable advice and kindness in offering their facilities for the collection of specimens. This work was supported by a Grant in Aid for Miscellaneous Scientific Research from the Ministry of Education of Japan (No. 60771487 and No. 62771442).

REFERENCES

BENDIX-ALMGREEN, S. E. (1983). *Carcharodon megalodon* from the upper Miocene of Denmark, with comments on elasmobranch tooth enameloid: coronoïn. *Bulletin of the Geological Society of Denmark*, **32**, 1–32.

EVERETT, M. M. & MILLER, W. A. (1981). Histochemistry of lower vertebrate calcified structures. 1. Enamel of the dogfish *Squalus acanthias* compared with mammalian enamel and homologous dentine. *Journal of Morphology* **170**, 95–111.

FOSSE, G., RISNES, S. & HOLMBAKKEN, N. (1974). Mineral distribution and mineralization pattern in the enameloid of certain elasmobranchs. *Archives of Oral Biology* **19**, 771–780.

GARANT, P. R. (1970). An electron microscopic study of the crystal-matrix relationship in the teeth of the dogfish *Squalus acanthias* L. *Journal of Ultrastructure Research* **30**, 441–449.

GOTO, M. (1978). Histogenetic studies on the teeth of leopard shark (*Triakis scyllia*). *Journal of the Stomatological Society, Japan* **45**, 527–584.

HEROLD, R. C., GRAVER, H. T. & CHRISTNER, P. (1980). Immunohistochemical localization of amelogenins in enameloid of lower vertebrate teeth. *Science* **207**, 1357–1358.

ISOKAWA, S., TSUBOUCHI, M., AOKI, K., IMAI, M., KAWAI, A. & TSUCHIDA, S. (1970). Studies on the developing enameloid of a fish (*Hoplognathus fasciatus*). 1. Mineralization pattern of enameloid matrix. *Journal of Nihon University School of Dentistry* **12**, 43–49.

KEMP, N. E. (1985). Ameloblastic secretion and calcification of the enamel layer in shark teeth. *Journal of Morphology* **184**, 215–230.

KEMP, N. E. & PARK, J. H. (1974). Ultrastructure of the enamel layer in developing teeth of the shark *Carcharhinus menisorrah*. *Archives of Oral Biology* **19**, 633–644.

KEMP, N. E. & WESTRIN, S. K. (1979). Ultrastructure of calcified cartilage in the endoskeletal tesserae of sharks. *Journal of Morphology* **160**, 75–102.

KEREBEL, B., DACULSI, G. & RENAUDIN, S. (1977). Ultrastructure des améloblastes au cours de la formation de l'émailoïde des sélaciens. *Biologie Cellulaire* **28**, 125–130.

KERR, T. (1955). Development and structure of the teeth in the dogfish, *Squalus acanthias*, L. and *Scyliorhynus caniculus* (L.). *Proceedings of the Zoological Society of London* **125**, 95–114.

KERR, T. (1960). Development and structure of some actinopterygian and urodele teeth. *Proceedings of the Zoological Society of London* **133**, 401–422.

MOSS, M. L. (1977). Skeletal tissues in sharks. *American Zoologist* **17**, 335–342.

NANCI, A., BRINGAS, P., JR., SAMUEL, N. & SLAVKIN, H. C. (1983). Selachian tooth development. III. Ultrastructural features of secretory amelogenesis in *Squalus acanthias*. *Journal of Craniofacial Genetics and Developmental Biology* **3**, 53–73.

PROSTAK, K., SEIFERT, P. & SKOBE, Z. (1988*a*). Ultrastructure of odontogenic cells during skate enameloid matrix formation. *Journal of Dental Research* **67**, 392.

PROSTAK, K., SEIFERT, P. & SKOBE, Z. (1988*b*), Inhibition of protein secretion by colchicine in skate tooth buds. *Journal of Dental Research* **67**, 392.

REIF, W.-E. (1979). Structural convergences between enameloid of actinopterygian teeth and of shark teeth. *Scanning Electron Microscopy* **II**, 547–554.

SASAGAWA, I. (1988). The appearance of matrix vesicles and mineralization during tooth development in three teleost fishes with well-developed enameloid and orthodentine. *Archives of Oral Biology* **33**, 75–86.

SLAVKIN, H. C., SAMUEL, N., BRINGAS, P., JR., NANCI, A. & SANTOS, V. (1983). Selachian tooth development. II. Immunolocalization of amelogenin polypeptides in epithelium during secretory amelogenesis in *Squalus acanthias*. *Journal of Craniofacial Genetics and Developmental Biology* **3**, 43–52.

TRAVIS, D. F. & GLIMCHER, M. J. (1964). The structure and organization of, and the relationship between the organic matrix and the inorganic crystals of embryonic bovine enamel. *Journal of Cell Biology* **23**, 447–497.

WAKITA, M., KOBAYASHI, M. & SHIOI, T. (1983). The decalcification method for electron microscopy using L-ascorbic acid. *Japanese Journal of Oral Biology* **25**, 691–699.